## Conclusions

Although a simple analytical tool, TLC continues to be an important screening and scouting method for almost all industries. The various TLC layers and supports discussed above are available worldwide and the large amount of literature available continues to show the versatility of this tool. These products allow the chromatographer a wide range of possibilities for attacking an analytical problem with some knowledge and the confidence that success will be attained with a few plates and a few solvent systems.

*See also:* **II/Chromatography: Thin-Layer (Planar):** Preparative Thin-Layer (Planar) Chromatography. **III/Impregnation Techniques: Thin-Layer (Planar) Chromatography. Silver Ion:** Thin-Layer (Planar) Chromatography.

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# Mass Spectrometry

**W. E. Morden,** LGC, Runcorn Research Centre, Cheshire, UK

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## Introduction

Thin-layer chromatography (TLC) and high performance thin-layer chromatography (HPTLC), collectively often referred to as planar chromatography, in combination with *in situ* mass spectrometry (MS), provides a reliable means for the unequivocal identification of substances separated by this type of chromatography. More specifically, the ability to obtain mass spectrometric information from the separated analytes directly from the TLC/HPTLC sorbent obviates the need to recover the analytes of interest prior to analysis. Although TLC-MS is a useful method of separation and detection, it does have its limitations where complex matrices are encountered. However, tandem mass spectrometric (MS-MS) techniques can be used to enhance the mass spectral data by reducing the interference caused by coeluting analytes or general background noise generated by excipients on the plate.

### Practical Considerations

Where the resolution of the separation is high, such as in capillary column chromatography, identification of components based on retention time is a well-established practice. Planar chromatography as normally practised does not achieve a separation efficiency equivalent to that obtained by column chromatography. Consequently, the identification of components separated by planar methods based solely on $R_F$ values and UV or fluoresence properties will always leave a higher degree of uncertainty about the true identity of the compounds present. Positive identification of unknowns has to be made by subjecting the analyte to a spectroscopic technique such as mass spectrometry.

As with other methods of chromatography, the use of MS as a TLC/HPTLC detector involves the detection and identification of the analyte in the presence of interfering materials from the chromatographic mobile and stationary phases. An analyte separated by planar techniques will be presented to a mass spectrometer along with the stationary phase, any organic phase modifier present, binding agents and fluorescent indicators. It is possible that residual solvents from the mobile phase, some of which may have high boiling points may be present. At best, the mass spectra obtained from a single spot taken from, for example, a silica gel HPTLC plate, will contain ions from the analyte with contributions from all these other compounds. When ionization is by fast atom bombardment (FAB) the situation is exacerbated by the formation of matrix and salt ions. Although useful information can be obtained from TLC/HPTLC-MS experiments, the information so obtained can be improved by using tandem mass spectrometric methods.

### Mass Spectrometric Benefits of Planar Separations

The advantages of HPTLC to the mass spectrometrist are equally important. The contribution made by the

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chosen detector in evaluating a separation method is often ignored. It is often assumed that the detector of choice is equally sensitive to all analytes presented to it and that the separation method and the detector do not influence each other. As a result, universal detectors that couple high sensitivity, specificity and selectivity are in great demand. A mass spectrometer is such a device. However, the operating limits of the mass spectrometer place some restraints on the chromatographic method of choice. Although the ionization of analytes may be considered as instantaneous, mass analysis is not. However, planar separations do not impose the same constraints on the mass spectrometer since the chromatography is completed prior to the analytes being presented to the mass spectrometer.

### Planar Separations as a Sample Clean-up Process

A further use of HPTLC is where the chromatography is simply a clean-up process, or a purification step. The planar separation can be used to remove impurities such as plasticizers or high boiling hydrocarbons. A solvent system can be used that will allow the migration of the contaminants up the plate but will leave the sample of interest at, or close to, the origin. The purified analyte can then be introduced into the mass spectrometer by one of the methods described below.

### Instrumentation

The approach to analysis by TLC/HPTLC-MS may be divided into two basic methods: instrumental and manual. The instrumental method involves the use of an automated probe onto which the whole chromatogram is attached. With this method, the integrity of the chromatogram is retained, but is restricted to ionization in the liquid phase by methods such as FAB and the related technique of liquid secondary ion mass spectrometry (LSIMS).

The manual technique involves the removal of the zone of interest from the plate. After removal, the analyte may be extracted from the sorbent prior to analysis, or analysed directly from the sorbent. The manual method is readily implemented and requires no further equipment or interfaces. Also, the classical vapour-phase electron and chemical methods of ionization (EI/CI) can be used.

### Sample Application

The most common method of application, and that most suited to MS applications when MS analysis is to be straight from the adsorbent, is by a glass capillary as a spot ('spotting'). For single MS experiments a single spot is usually sufficient. Where multi-MS experiments are to be performed, such as when tandem mass spectrometry is to be used, the application of a row of replicate spots is the preferred method, the separated analytes from a fresh spot being used as those from a previous one become exhausted. If the analytes are to be removed from the adsorbent before analysis by MS the application of a short line ('streaking') has been found to be the best method. Whether the *spotting* or *streaking* method is used, care is needed during application to keep the analyte origin as compact as possible. A sample that is too large causes poor-quality separations due to the analyte diffusing and causing the spot to broaden. Quantities of sample spotted are in the range of 1–50 $\mu g\ \mu L^{-1}$, 1–10 $\mu L$ being applied to the plate by multiloading a 1–5 $\mu L$ capillary.

Care has to be taken not to damage the surface of the adsorbent as this causes the mobile phase to elute unevenly, resulting in distorted spots and so leading to a loss of chromatographic resolution. The type of matrices being studied and the detection limit of the mass spectrometer are then the critical factors in determining to what extent analysis is possible. However, when sample loading needs to be high, the deconvolution-by-mass capabilities of the mass spectrometer often compensate for the reduction in resolution.

### Sample Introduction after Planar Separation

Although analysis by planar chromatographic techniques and mass spectrometry has been practised for many years, there remain three principal methods of sample introduction.

The first method involves the analyte of interest being recovered from the adsorbent by elution with an appropriate solvent and introduced into the mass spectrometer's ion source as a discrete sample. After the area of interest of the chromatogram has been identified, usually by visual means, the perimeter of the area of interest, the 'spot', is marked with a soft-leaded pencil. The adsorbent is loosened from the backing with a flat-bladed tool such as a spindle screwdriver or microspatula. The loosened material is removed from the plate and mixed with a small quantity of solvent. Ultrasonification can assist in the extraction process. A small plug of quartz wool packed into a drawn-out Pasteur pipette (**Figure 1**) acts as a filter medium. The filtered adsorbent is washed with one aliquot of solvent.

Depending upon the method of ionization to be used, the extracted material may be introduced into the ion source by using the direct insertion probe for the ionization of volatile anlaytes by EI/CI; or by a desorption probe or cassette, such as for analysis by

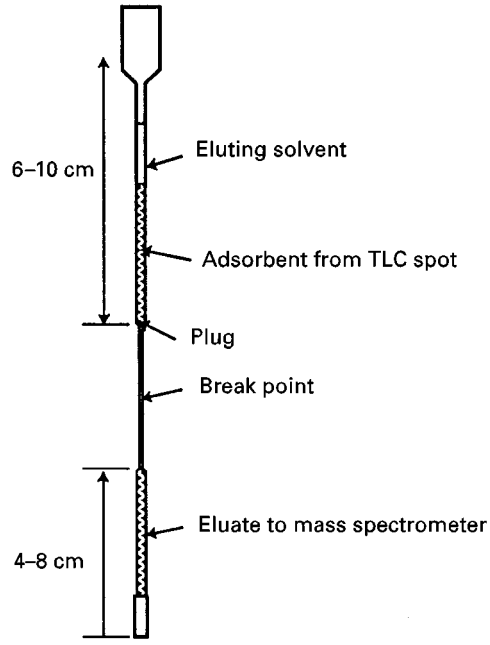


**Figure 1** Analyte recovery by solvent elution.

FAB, LSIMS or MALDI (matrix-assisted laser desorption ionization), for the ionization of polar, non-volatile and/or thermally labile analytes. This method of sample introduction is particularly useful when the sample application methods of multispotting or streaking methods are used to obtain sufficient analyte for multi-MS-MS experiments.

In the second method the analyte and adsorbent are not separated. Both are introduced into the ion source simultaneously. After visual location, the spot is scraped off the plate and introduced into the ion source by one of the methods described above.

Both of the above methods destroy the integrity of the chromatogram. A third method of introduction uses a dedicated probe that enables the whole chromatogram to be introduced into the FAB/LSIMS ion source, so that a spatially resolved total ion current chromatogram of the organic material on the adsorbent may be obtained. With this type of probe it is necessary to use aluminium-backed plates. A strip approximately 10 mm × 65 mm, which is essentially one track of a developed plate, is cut out and mounted on the end of the probe. An appropriate matrix is painted down the length of the strip and the chromatogram is driven at a constant rate, with a maximum of 50 mm $min^{-1}$, by a stepping motor. The pulses to the stepping motor are controlled in conjunction with the scan control of the mass spectrometer, with the result that the whole chromatogram is scanned by the FAB/LSIMS beam. Mass spectral information is acquired for the whole chromatogram. The analysis is analogous to the column chromatography experiment in that the analytes are ionized sequentially throughout the chromatogram.

### Sample Concentration

As the HPTLC plate develops, spot diffusion occurs with the result that what was 0.5–1 mm spot at the origin may well be 5 or 6 mm in diameter after complete development. Thus the analyte may well be distributed over 10 times more adsorbent than when at the origin, with the result that when the analytes are introduced into the ion source along with the adsorbent and other extraneous compounds from the plate there is considerably more adsorbent than analyte presented for ionization. Because there is a finite amount of material that can be introduced into the ion source, occasions may arise where there is insufficient analyte available from which good quality mass spectral data may be obtained. Several methods of sample concentration have been devised to overcome this natural spot diffusion phenomenon. For example, an analyte spot can be encircled with a ring of solvent. As the solvent diffuses through the adsorbent the analyte will slowly migrate to, and concentrate at, the centre of the spot. The concentrated spot can then be removed and analysed.

An alternative is where, after chromatographic development on aluminium- and plastic-backed plates, the edge of the plate may be trimmed off using dressmaker's pinking shears. This leaves the plate with a regular saw-toothed edge. The plate is rotated by 180° followed by the application of solvent. The analytes are thus concentrated into the tips of the triangular-shaped segments of the plate. Once concentrated, the zone may be removed from the plate and introduced into the ion source as previously described.

A general method not confined to aluminium- and plastic-backed plates involves scribing a trapezoidal line around the sample spot (**Figure 2**). A strip, 1–2 mm wide, of the adsorbent on the outside of the

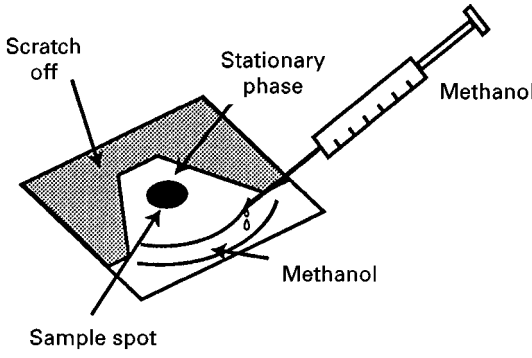


**Figure 2** Zone concentration.

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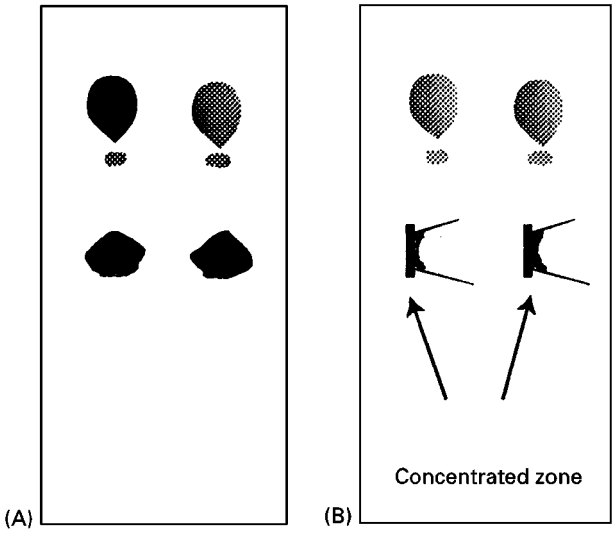


**Figure 3** Zones (A) before and (B) after concentration.

line is removed. Then, from a 100 μL syringe, a quantity of an eluting solvent is slowly deposited on the base of the spot. After several tens of seconds the analyte condenses at the top of the trapezoid (**Figure 3**). The concentrated analyte and adsorbent are removed from the plate and are introduced into the ion source as described previously.

This method of spot concentration completely overcomes the need to overload the plate, with associated loss of chromatographic resolution, to enable sufficient sample to be introduced into the mass spectrometer for ionization. It is highly satisfactory prior to both vapour-phase and liquid-phase ionization.

## Obtaining Mass Spectral Data

### Vapour-Phase Ionization of the Analytes Directly from the Adsorbent

Where the analytes are volatile and thermally stable and are to be ionized by EI/CI, the zone of interest is concentrated as described earlier and a portion of the adsorbent is placed in a standard quartz sample tube. A small plug of quartz wool is placed on top of the sample to prevent the fine silica being sucked out of the tube during evacuation. The sample tube is then fitted to the direct insertion probe. The volatile analyte is desorbed by heat from the involatile adsorbent.

Where ionization is by MALDI, various methods have been described that enable mass spectra to be obtained directly from the TLC plate. TLC-MALDI takes advantage of the ability of MALDI to ionize fragile, low and high molecular weight compounds with a high degree of sensitivity and without significant fragmentation.

### Liquid-Phase Ionization of the Analytes Directly from the Adsorbent

For polar, nonvolatile analytes, the spot is concentrated as described above. The adsorbent is then removed from the plate and slurried directly on the desorption probe with the chosen matrix.

### Reducing Surface Effects during FAB/LSIMS Ionization

When compounds with high surface activity properties are present in a mixture, even as minor components, they have been shown to suppress the ionization of the major components during analysis by FAB/LSIMS-MS. Compounds such as surfactants possess these properties to such an extent that analysis by liquid phase inonization is unpredictable. Often the major component (of interest) is suppressed so much that it may not appear to be the major component at all. Planar chromatography has been shown to be an effective way of separating such suppressants from the analyte of interest.

## Solo Mass Spectrometer versus Tandem Mass Spectrometry

As described previously, the use of MS as a TLC/HPTLC detector involves the detection and identification of the analyte in the presence of interfering compounds from the chromatographic process. At best, the mass spectra obtained from a single spot after planar separation will contain ions from the analyte and from these other compounds. When ionization is by FAB/LSIMS the situation may be exacerbated by the formation of matrix and salt ions (**Figure 4**). Although useful information can be obtained from TLC/HPTLC-MS

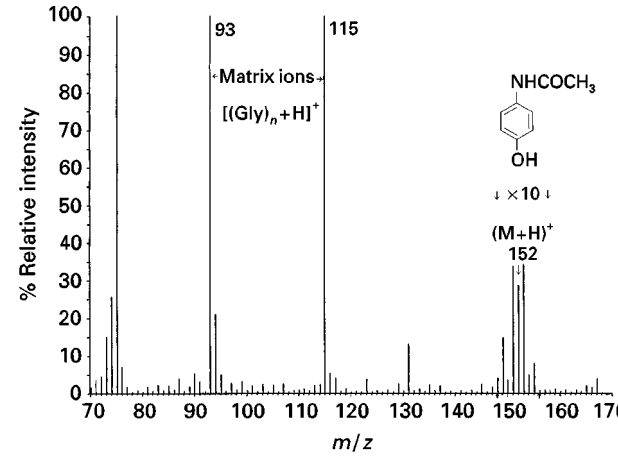


**Figure 4** FAB-MS spectrum of paracetamol obtained from urine following ascending chromatography on diol bonded phase.

experiments, the information so obtained can be improved by using tandem mass spectrometric methods.

Tandem mass spectrometry is now a well-established analytical technique. Tandem mass spectrometry offers advantages in three major areas: structure elucidation of organic and biological molecules; the identification of components in complex matrices with or without chromatographic separation; and in trace analysis.

Several types of instrument capable of performing MS-MS analyses are available, the two most common types being the triple-quadrupole and the sector/quadrupole hybrid. In both types of instrument the ions selected in the first analyser are transmitted into the reaction region, normally a gas cell, where fragmentation occurs by collisions with a target gas, to yield characteristic product ions to be analysed and detected by the second analyser (**Figure 5**).

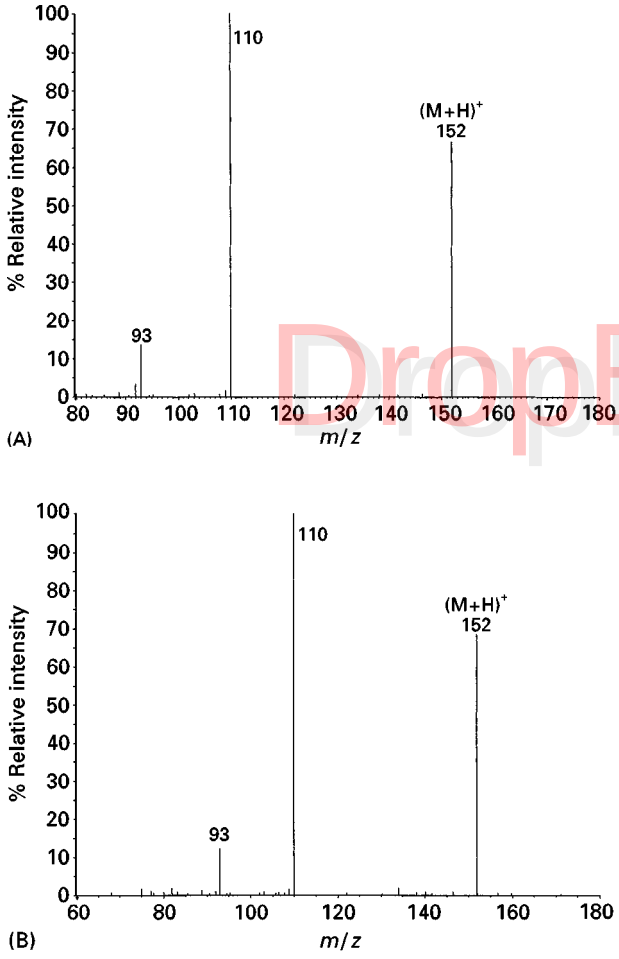


**Figure 5** (A) FAB-MS-MS collision induced dissociation product ion spectrum of the $(M + H)^+$ ion from a standard sample of paracetamol following ascending chromatography on diol bonded phase. Collision energy, 60–40 eV; collision gas, argon at an indicated pressure of $3.0 \times 10^{-6}$ mbar. (B) FAB-MS-MS collision induced dissociation spectrum of the $(M + H)^+$ ion from paracetamol extracted from urine and following ascending chromatography on diol bonded phase. Collision energy, 60–40 eV; collision gas, argon at an indicated pressure of $3.0 \times 10^{-6}$ mbar.

### Ionization in Tandem Mass Spectrometry

Although all of the currently available methods of ionization are applicable to tandem mass spectrometry, where planar separations are involved the choice is limited to EI/CI and FAB/LSIMS. Although electron ionization (EI) is still the most commonly encountered method of ionization, and is used extensively in MS-MS studies, ionization methods such as FAB/LSIMS and CI, which normally produce intense ions providing molecular weight information, are preferable. It is desirable to produce as few ions of different $m/z$ values as possible when using MS-MS following planar separations.

The benefits of FAB/LSIMS and CI are two-fold. First, they usually produce a spectrum with fewer interferences, enabling easier selection of precursor ion. Second, an increase in sensitivity is often achieved if all the ions current obtained from an analyte is concentrated in one ion.

### Ion Dissociation in Tandem Mass Spectrometry

Although the MS-MS instrumentation is extensive, there is only one basic principle: the measurement of the mass-to-charge ratios of ions before and after dissociation in the reaction region. The change in mass of an ion carrying a single charge is the most commonly measured process and can be represented by the following equation:

$$M_p^+ \rightarrow M_d^+ + M_n$$

where $M_p^+$ represents the precursor ion, $M_d^+$ represents the product ion and $M_n$ represents the neutral fragment. The precursor ion may be the ion representing the relative mass of the species but need not necessarily be so; the precursor ion could be any ion formed within the mass spectrometer. However, molecular ions, and ions generated within the ion source, are the major subjects of MS-MS experiments. The product ion, $M_d^+$, represented in the above equation may be one of many fragment ions formed by simultaneous dissociation within the reaction region, and as such indicates a direct relationship between it and the precursor ion. The neutral fragment $M_n$, being uncharged, is not normally detected. However, since $M_p^+$ and $M_d^+$ are measured the mass of the neutral fragment may be calculated by difference. These three variables form the basis of most MS-MS experiments and it is this basis that makes tandem mass spectrometry such a powerful analytical tool.

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### Scan Functions in Tandem Mass Spectrometry

Although there are several scan functions that can be performed by tandem instruments, the most commonly used function in TLC/HPTLC applications is the product ion scan. The analytical characteristics of TLC/HPTLC-MS-MS product ion analysis contrast it with analysis by chromatography–chromatography–mass spectrometer.

Even so, just as with conventional MS, tandem mass spectrometry has its limitations. MS-MS cannot easily differentiate between isomeric and isobaric species. Isomers tend to produce similar product ion spectra and, since they cannot be separated by mass, two isomers transmitted into the gas cell simultaneously will give a product ion spectrum that is representative of the sum of the individual compounds.

Similarly, if isobaric components of the same elemental composition but of different structure are focused by the first analyser, transmitted into the gas cell and dissociated by collisions, the product ion spectrum obtained from the second analyser will be a compilation of product ions from the two components. It can be seen, therefore, that some form of chromatographic separation such as TLC/HPTLC prior to ionization can enhance the separative and characterization powers of the tandem mass spectrometer. Conversely, the use of MS-MS can greatly enhance the identification and characterization of isomeric and isobaric species separated by planar chromatography.

## Conclusions

The use of MS and MS-MS as methods of detection in TLC and HPTLC separations is now well established and has been shown to be a powerful tool in the analyst's armoury. In practice, HPTLC-MS-MS has been shown to be no more technically demanding than traditional TLC-MS, yet the information provided is more detailed. Structurally important diagnostic ions are more easily observed by the elimination of matrix ions and general background clutter. Planar separation should be considered as a complementary technique alongside column methods of chromatographic separation where detection is by mass spectrometric methods. It can be applied to a wide range of polar and nonpolar analytes and is amenable to vapour-phase and liquid-phase methods of ionization. Moreover, where ionization is by laser desorption, separation by planar techniques may be the only choice.

*See also:* **I/Mass Spectrometry.**

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